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=> s method and graft rejection
L1        17086 METHOD AND GRAFT REJECTION

=> s l1 and TGF beta
L2          146 L1 AND TGF BETA

=> s l2 and peripheral mononuclear blood cell
   4 FILES SEARCHED...
L3            0 L2 AND PERIPHERAL MONONUCLEAR BLOOD CELL

=> s l2 and irradiation
L4            0 L2 AND IRRADIATION

=> s l1 and irradiation
L5          459 L1 AND IRRADIATION

=> s l5 and ex vivo
L6           11 L5 AND EX VIVO

=> s l6 and TGF beta
L7            0 L6 AND TGF BETA

=> dup remove l6
PROCESSING COMPLETED FOR L6
L8            8 DUP REMOVE L6 (3 DUPLICATES REMOVED)

=> d l8 1-8 cbib abs

L8   ANSWER 1 OF 8  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:310359  Document No.: PREV200200310359. The infusion of ex
     vivo activated and expanded CD4+CD25+ immune regulatory cells
     inhibits graft-versus-host disease lethality. Taylor, Patricia A.; Lees,
     Christopher J.; Blazar, Bruce R. (1). (1) Department of Pediatrics,
     Division of Bone Marrow Transplantation, University of Minnesota Cancer
     Center, Minneapolis, MN, 55455: blaza001@tc.umn.edu USA. Blood, (May 15,
     2002) Vol. 99, No. 10, pp. 3493-3499. http://www.bloodjournal.org/. print.
     ISSN: 0006-4971. Language: English.
AB   Immune regulatory CD4+CD25+ cells play a vital role in the induction and
     maintenance of self-tolerance and the prevention of autoimmunity.
     Recently, CD4+CD25+ cells have been shown to be required for the
     ex vivo induction of tolerance to alloantigen via
     costimulatory blockade and to inhibit allogeneic skin graft
     rejection. Data presented here demonstrate that CD4+CD25+ cells
     play an important role in graft-versus-host disease (GVHD) generation.
     Depletion of CD4+CD25+ cells from the donor T-cell inoculum or in vivo
     CD25-depletion of the recipient before transplantation resulted in
     increased GVHD mediated by CD4+ or whole T cells in several strain
     combinations irrespective of the total body irradiation
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conditioning regime. The infusion of freshly purified donor CD4+CD25+ cells modestly inhibited GVHD when administered in equal numbers with whole CD4+ cells. Because CD4+CD25+ cells only account for 5% to 10% of the total CD4+ population, the administration of high numbers of fresh donor CD4+CD25+ cells may not be clinically practical. However, we found that large numbers of CD4+CD25+ cells can be obtained by **ex vivo** activation and expansion. Cultured CD4+CD25+ cells, administered in equal numbers with CD4+ T cells or CD25-depleted whole T cells, resulted in significant inhibition of rapidly lethal GVHD. To our knowledge, this study is the first to demonstrate that activated, cultured CD4+CD25+ cells can offer substantial protection in a relevant in vivo animal model of disease. These data have important ramifications for clinical bone marrow and solid organ transplantation. CD4+CD25+ cells warrant consideration as an exciting new modality of cellular therapy for the inhibition of undesirable autologous and allogeneic responses.

L8 ANSWER 2 OF 8 MEDLINE
2001446208 Document Number: 21384685. PubMed ID: 11493272. Amplification of engrafted hepatocytes by preparative manipulation of the host liver.
Guha C; Deb N J; Sappal B S; Ghosh S S; Roy-Chowdhury N; Roy-Chowdhury J.
(Department of Radiation Oncology, The Marion Bessin Liver Research Center Departments of Medicine and Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, U.S.A.) ARTIFICIAL ORGANS, (2001 Jul) 25 (7) 522-8. Ref: 25. Journal code: 7802778. ISSN: 0160-564X. Pub. country: United States. Language: English.

AB Scarcity of donor livers is a major obstacle to the general application of hepatocytes for the development of bioartificial liver assist devices as well as intracorporeal engraftment of hepatocytes for the treatment of inherited metabolic diseases. The number of hepatocytes that can be transplanted into the liver safely in a single sitting also limits the utility of this procedure. These limitations could be addressed by providing preferential proliferative advantage to the transplanted cells. Studies using transgenic mouse recipients or donors have indicated that massive repopulation of the host liver by engrafted hepatocytes requires that the transplanted cells are subjected to a proliferative stimulus to which the host hepatocytes cannot respond. Prevention of host hepatocyte proliferation has been achieved by treatment with a plant alkaloid, retrorsine. Because retrorsine is carcinogenic, we have evaluated preparative **irradiation** for this purpose. The proliferative stimulus may consist of the loss of hepatic mass (e.g., partial hepatectomy, reperfusion injury or induction of Fas-mediated apoptosis by gene transfer) or administration of stimulants of hepatocellular mitosis (e.g., growth factors or thyroid hormone). Potential applications of these preparative manipulations of the host liver include the treatment of inherited metabolic disorders by transplantation of allogeneic hepatocytes, hepatocyte-mediated **ex vivo** gene therapy, rescuing liver cancer patients from radiation-induced liver damage, and expansion of human hepatocytes in animal livers.

L8 ANSWER 3 OF 8 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
2001013514 EMBASE Immunomodulation for intestinal, transplantation by allograft **irradiation**, adjunct donor bone marrow infusion, or both. Murase N.; Ye Q.; Nalesnik M.A.; Demetris A.J.; Abu-Elmagd K.; Reyes J.; Ichikawa N.; Okuda T.; Fung J.J.; Starzl T.E.. Dr. N. Murase, Department of Surgery, E1555 Biomedical Science Tower, University of Pittsburgh, Pittsburgh, PA 15213, United States. murase+@pitt.edu. Transplantation 70/11 SUPPL. (1632-1641) 15 Dec 2000.
Refs: 48.
ISSN: 0041-1337. CODEN: TRPLAU. Pub. Country: United States. Language: English. Summary Language: English.

AB Background. The passenger leukocytes in the intestine have a lineage profile that predisposes to graft-versus-host disease (GVHD) in some animal models and have inferior tolerogenic qualities compared with the

leukocytes in the liver, other solid organs, and bone marrow. Elimination by **ex vivo irradiation** of mature lymphoid
elements from the bowel allografts is known to eliminate the GVHD risk. We hypothesized that infusion of donor bone marrow cells (BMC) in recipients of irradiated intestine would improve tolerogenesis without increasing the risk of GVHD. **Methods**. Orthotopic small intestine
transplantation was performed with the GVHD-prone Lewis (LEW)-to-Brown Norway (BN) combination and the reverse GVHD-resistant BN-to-LEW model under a short course of tacrolimus treatment (1 mg/kg/day, days 0-13, 20, 27). Grafts were irradiated **ex vivo**, using a (137)Cs
source. In selected experimental groups, donor BMC (2.5 x 10(8)) were infused on the day of small intestine transplantation. Results. The unmodified LEW intestine remained intact, whether transplanted alone or with adjunct donor BMC infusion, but all of the BN recipients died of GVHD after approximately 2 months. Intestinal graft **irradiation** (10
Gy) effectively prevented the GVHD and prolonged survival to 92.5 days, but all of the BN recipients died with chronic rejection of the LEW grafts, which was prevented by infusion of adjunct donor BMC without causing GVHD. In the GVHD-resistant reverse strain direction (BN .fwdarw. LEW), all intestinal recipients treated for 27 days with tacrolimus survived .gtoreq.150 days without regard for graft **irradiation**
or adjunct BMC, but chronic rejection was severe in the irradiated intestine, moderate in the unaltered graft, and least in the irradiated intestine transplanted with adjunct BMC. Mild arteritis in the 150 day allografts of both strain combinations (i.e., LEW .fwdarw. BN and BN .fwdarw. LEW) may have been **irradiation** associated, but this was prevented when weekly doses of tacrolimus were continued for the duration of the experiment rather than being stopped at 27 days. Conclusions. Recipients are protected from GVHD by irradiating intestinal allografts, but the resulting leukocyte depletion leads to chronic rejection of the transplanted bowel The chronic rejection is prevented with adjunct donor BMC without causing GVHD. Although application of the strategy may be limited by the possibility of radiation injury, the results are consistent with the paradigm that we have proposed to explain organ-induced graft acceptance, tolerance, and chronic rejection.

L8 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:297571 Document No.: PREV200100297571. Adoptive immunotherapy with fludarabine-treated cytomegalovirus primed donor splenocytes following MHC mis-matched allogeneic bone marrow transplantation in mice. Roback, John D. (1); Mittelstaedt, Stephen (1); Hillyer, Christopher D. (1); Waller, Edmund K. (1). (1) Pathology and Laboratory Medicine and Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 176a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology. ISSN: 0006-4971. Language: English. Summary Language: English.

AB Background: Delayed immune reconstitution among recipients of T-cell depleted (TCD) allogeneic hematopoietic cell transplants predisposes to lethal infections, including cytomegalovirus (CMV). In order to study antiviral cellular immunity in this setting, we have characterized CMV infections after syngeneic and MHC mis-matched marrow transplantation in mice. Study Design: BMT recipients (Balb/C X C57BL/6 F1 mice; 6-8 weeks old) were conditioned with 11 Gy total body **irradiation**. Bone
marrow (BM), obtained from either F1, Balb/c, or C57BL/6 donors, was T-cell depleted (TCD) using miniMACS columns and 5X106 TCD BM cells were infused IV into recipients. Some recipients received simultaneous infusions of 107 donor splenocytes (either immune or non-immune) which were either pretreated with fludarabine (55 muM/ml) or gamma-
irradiation (7.5 Gy). 2 hours after BM and splenocyte infusion, mice were injected IP with murine CMV (MCMV, Smith strain) at selected doses (102-5X106 PFU; n=5-15 mice/group) and then monitored closely for morbidity and mortality. Results: Without BMT 80% of irradiated mice die,

while F1 mice receiving TCD BMT from either F1, Balb/C or C57BL/6 mice have 100% survival. C57BL/6>F1 and Balb/C>F1 mice were more susceptible to CMV infection than F1>F1 mice, with LD50s of 8.1X102 ($p<0.05$), 7.4X104, and 2.7X105 PFU, respectively. C57BL/6>F1 mice infused with fludarabine-treated splenocytes from immune C57BL/6 donors prior to infection with 104 CMV displayed 100% survival (14/14) compared to 50% (5/10) survival in the non-splenocyte treated controls ($p<0.05$). No significant GvHD was seen among recipients of fludarabine-treated splenocytes. In preliminary analysis, splenocytes from non-immune donors did not significantly improve survival. Conclusions: Parent strain>F1 BMT allows the antiviral and GvHD effects of MHC mis-matched donor splenocytes to be examined in the absence of **graft rejection**. While MHC mis-matched C57BL/6>F1 TCD BMT was associated with significantly increased susceptibility to CMV infection post-transplantation, CMV mortality was prevented by adoptive immunotherapy with fludarabine-treated splenocytes from immune C57BL/6 donors. **Ex vivo** treatment of donor T-cells with fludarabine is a novel **method** of adoptive immunotherapy without GvHD activity that may be useful among recipients of T-cell depleted allografts.

L8 ANSWER 5 OF 8 MEDLINE DUPLICATE 2
1999265711 Document Number: 99265711. PubMed ID: 10334542. Improved outcome with T-cell-depleted bone marrow transplantation for acute leukemia. Aversa F; Terenzi A; Carotti A; Felicini R; Jacucci R; Zei T; Latini P; Aristei C; Santucci A; Martelli M P; Cunningham I; Reisner Y; Martelli M F. (BMT Program, Section of Hematology and Clinical Immunology, University of Perugia, Italy.. aversa@unipg.it) . JOURNAL OF CLINICAL ONCOLOGY, (1999 May) 17 (5) 1545-50. Journal code: 8309333. ISSN: 0732-183X. Pub. country: United States. Language: English.

AB PURPOSE: To eliminate the risk of rejection and lower the risk of relapse after T-cell-depleted bone marrow transplants in acute leukemia patients, we enhanced pretransplant immunosuppression and myeloablation. PATIENTS AND **METHODS:** Antithymocyte globulin and thiotepa were added to standard total-body **irradiation**/cyclophosphamide conditioning. Donor bone marrows were depleted **ex vivo** of T lymphocytes by soybean agglutination and E-rosetting. This approach was tested in 54 consecutive patients with acute leukemia who received transplants from HLA-identical sibling donors or, in two cases, from family donors mismatched at D-DR. No posttransplant immunosuppressive treatment was given as graft-versus-host disease (GVHD) prophylaxis. RESULTS: Neither **graft rejection** nor GVHD occurred. Transplant-related deaths occurred in six (16.6%) of 36 patients in remission and in seven (38.8%) of 18 patients in relapse at the time of transplantation. The probability of relapse was .12 (95% confidence interval [CI], 0 to .19) for patients with acute myeloid leukemia and .28 (95% CI, .05 to .51) for patients with acute lymphoblastic leukemia who received transplants at the first or second remission. At a median follow-up of 6.9 years (minimum follow-up, 4.9 years), event-free survival for patients who received transplants while in remission was .74 (95% CI, .54 to .93) for acute myeloid leukemia patients and .59 (95% CI, .35 to .82) for acute lymphoblastic leukemia patients. All surviving patients have 100% performance status. CONCLUSION: Adding antithymocyte globulin and thiotepa to the conditioning regimen prevents rejection of extensively T-cell-depleted bone marrow. Even in the complete absence of GVHD, the leukemia relapse rate is not higher than in unmanipulated transplants.

L8 ANSWER 6 OF 8 MEDLINE
1999176459 Document Number: 99176459. PubMed ID: 10078573. Prevention of autoimmune recurrence and rejection by adenovirus-mediated CTLA4Ig gene transfer to the pancreatic graft in BB rat. Uchikoshi F; Yang Z D; Rostami S; Yokoi Y; Capocci P; Barker C F; Naji A. (Department of Surgery, University of Pennsylvania Medical Center, Philadelphia, USA.) DIABETES, (1999 Mar) 48 (3) 652-7. Journal code: 0372763. ISSN: 0012-1797. Pub.

country: United States. Language: English.

AB Type 1 diabetes is the result of a selective destruction of pancreatic islets by autoreactive T-cells. Therefore, in the context of islet or pancreas transplantation, newly transplanted beta-cells are threatened by both recurrent autoimmune and alloimmune responses in recipients with type 1 diabetes. In the present study, using spontaneously diabetic BB rats, we demonstrate that whereas isolated islets are susceptible to autoimmune recurrence and rejection, pancreaticoduodenal grafts are resistant to these biological processes. This resistance is mediated by lymphohematopoietic cells transplanted with the graft, since inactivation of these passenger cells by **irradiation** uniformly rendered the pancreaticoduodenal grafts susceptible to recurrent autoimmunity. We further studied the impact of local immunomodulation on autoimmune recurrence and rejection by **ex vivo** adenovirus-mediated CTLA4Ig gene transfer to pancreaticoduodenal grafts. Syngeneic DR-BB pancreaticoduodenal grafts transduced with AdmCTLA4Ig were rescued from recurrent autoimmunity. In fully histoincompatible LEW-->BB transplants, in which rejection and recurrence should be able to act synergistically, AdmCTLA4Ig transduced LEW-pancreaticoduodenal allografts enjoyed markedly prolonged survival in diabetic BB recipients. In situ reverse transcription-polymerase chain reaction revealed that transferred CTLA4Ig gene was strongly expressed in both endocrine and exocrine tissues on day 3. These results indicate the potential utility of local CD28-B7 costimulatory blockade for prevention of alloimmune and autoimmune destruction of pancreatic grafts in type 1 diabetic hosts.

L8 ANSWER 7 OF 8 MEDLINE
2001170749 Document Number: 21088563. PubMed ID: 11271224. Effects of PUVA therapy on kidney allografts: results of a randomized prospective double-blind study. Kaden J; Oesterwitz H; May G; Strobelt V; Schroder K; Bohnke C; Gellert S; Groth J; Schabel J; Eismann R; Templin R; Sehland J. (Friedrichshain Hospital, Laboratory of Immunology, Berlin, Germany.) TRANSPLANT INTERNATIONAL, (1994) 7 Suppl 1 S275-80. Journal code: 8908516. ISSN: 0934-0874. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB After successful experimental organ transplant studies on the efficacy of PUVA therapy combining donor pretreatment with the photosensitizer 8-methoxypsoralen (P) and the **ex vivo** **irradiation** of organs with long-wave ultraviolet light (UVA) prior to transplantation, we started in 1989 the first randomized, prospective, double-blind study to clarify the efficacy of PUVA therapy in human kidney transplantation. This study included 50 kidney donors, 25 of whom were PUVA-treated. A total of 75 kidneys were transplanted in Berlin, Halle and Rostock. The complete data of these 75 recipients were available for the final evaluation. The PUVA group (n = 36) and the non-PUVA group (n = 39) were not statistically significantly different as to donor and recipient data. Regarding the results, no differences were seen in initial hospitalization time, early graft function, rejection rate, number and time of rejection episodes. After a follow-up of 24 months, both graft survival (PUVA vs. non-PUVA: 75% vs. 71.8%) and patient survival (97.2% vs. 97.4%, respectively) were comparably high. PUVA therapy did not influence the development of vascular rejection. Interestingly, the rate of late graft loss after the 6th posttransplant month was lower, but not statistically significantly so, in the PUVA than in the non-PUVA-group (2 vs. 6 graft losses). Thus, PUVA-pretreated kidneys may be associated with a reduced development of chronic rejection.

L8 ANSWER 8 OF 8 MEDLINE
88321983 Document Number: 88321983. PubMed ID: 3413649. Effect of pretransplant graft **irradiation** on canine intestinal transplantation. Williams J W; McClellan T; Peters T G; Nag S; Dean P; Banner B; Vera S R; Stenz F. (Department of Surgery, Rush-Presbyterian-St. Luke's Hospital, Chicago, Illinois 60612.) SURGERY, GYNECOLOGY AND

OBSTETRICS, (1988 Sep) 167 (3) 197-204. Journal code: 0101370. ISSN: 0039-6087. Pub. country: United States. Language: English.

AB This study was done to define the tolerance of **ex vivo** administered **irradiation** to intestinal allograft and to assess the effect of **irradiation** on the incidence and severity of rejection and graft versus host disease after intestinal transplantation in dogs. Excessive intestinal damage was produced by 2,500 rads, but 750 and 1,500 rads produced no detectable acute or chronic damage in dogs observed from 100 days to two years. Using cyclosporine for postoperative immunosuppression, 1,500 rads reduced the incidence of acute ($p = 0.05$) and chronic rejection ($p = 0.08$), yet did not impair intestinal absorption of cyclosporine. The greatest improvement in survival occurred with 750 rads ($p = 0.02$). Histologic evidence of graft versus host disease appeared in the native small intestine in two of four long term surviving dogs receiving a nonirradiated graft but in none of the dogs receiving irradiated grafts. **Irradiation** of the graft may be a promising adjunct in the search for a clinically applicable **method** of intestinal transplantation.

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=> s l1 and "PMBC"
L9              0 L1 AND "PMBC"

=> s l1 adn isolating mononuclear cell
MISSING OPERATOR L1 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l1 and isolating mononuclear cell
   3 FILES SEARCHED...
L10             0 L1 AND ISOLATING MONONUCLEAR CELL

=> d his

     (FILE 'HOME' ENTERED AT 09:17:53 ON 21 AUG 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:18:19 ON
     21 AUG 2002
L1          17086 S METHOD AND GRAFT REJECTION
L2            146 S L1 AND TGF BETA
L3              0 S L2 AND PERIPHERAL MONONUCLEAR BLOOD CELL
L4              0 S L2 AND IRRADIATION
L5            459 S L1 AND IRRADIATION
L6             11 S L5 AND EX VIVO
L7              0 S L6 AND TGF BETA
L8              8 DUP REMOVE L6 (3 DUPLICATES REMOVED)
L9              0 S L1 AND "PMBC"
L10             0 S L1 AND ISOLATING MONONUCLEAR CELL

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PROCESSING COMPLETED FOR L2
L11           105 DUP REMOVE L2 (41 DUPLICATES REMOVED)

=> s l11 and IL2
L12             1 L11 AND IL2

=> d l12 cbib abs

L12  ANSWER 1 OF 1  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:493318  Document No.: PREV200100493318. Real-time polymerase chain
     reaction analysis reveals an evolution of cytokine mRNA production in
     allograft acceptor mice. Xia, Dongyuan (1); Sanders, Aimee; Shah, Manisha;
     Bickerstaff, Alice; Orosz, Charles. (1) Ohio State University Transplant,
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410 W. 10th Avenue, N943 Doan Hall, Columbus, OH, 43210: xia-1@medctr.osu.edu USA. Transplantation (Baltimore), (September 15, 2001) Vol. 72, No. 5, pp. 907-914. print. ISSN: 0041-1337. Language: English. Summary Language: English.

AB Background: The relative contribution of pro-inflammatory and anti-inflammatory cytokines in promoting the rejection or acceptance of experimental cardiac allografts remains controversial. We hypothesized that the posttransplantation induction of a new immune response to graft alloantigens at a distant delayed-type hypersensitivity (DTH) site would force the immune system to reveal its current disposition toward graft alloantigen as it initiates the new immune response. Thus, we should be able to monitor the evolution of the immunologic relationship between allograft recipients and their grafts at any time posttransplantation by challenging the recipient for DTH responses to donor alloantigen and evaluating the cytokine profiles displayed at the DTH site. **Methods:** We have used the sensitive and quantitative technique of real-time polymerase chain reaction to evaluate the patterns of donor alloantigen-induced cytokine mRNA production for interleukin (IL)-2, interferon (IFN)-gamma, IL-4, IL-10, and transforming growth factor (**TGF**)**-beta**. We evaluated cytokine mRNA expression in cardiac allografts and in donor alloantigen-challenged DTH sites in mice that have either accepted or rejected cardiac allografts. Results: We observed the following. (1) Normal hearts and pinnae exhibited detectable baseline production of cytokine mRNAs: **TGF-beta** >IFN-gamma=IL-10>**IL2**->IL-4. (2) Both the accepted and rejecting cardiac allografts produced increased amounts of all cytokine mRNAs tested and displayed few quantitative differences in cytokine mRNA production. Notably, accepted allografts displayed enhanced IL-10 mRNA production on day 7 posttransplantation, but not on day 60 posttransplantation and reduced IFN-gamma mRNA production on day 60, but not day 7. (3) There was a high degree of variability in production levels among the various cytokine mRNAs, both for background levels and for allograft-stimulated levels. (4) Donor-reactive DTH sites of allograft rejector mice displayed a broad array of cytokine mRNAs, whereas the DTH sites of allograft acceptor mice displayed only IL-4 mRNA production. (5) Provision of exogenous **TGF-beta** or IL-10 at a DTH challenge site of allograft rejector mice caused a shift in the cytokine mRNA profile that minimized IFN-gamma and IL-2 mRNA production but spared IL-4, IL-10, and **TGF-beta** mRNA production. Conclusions: A broad array of cytokine mRNAs may be stockpiled for future use in cardiac allografts, regardless of whether the grafts will be accepted or rejected. This stockpile is continuously replenished for as long as the graft survives, thereby obscuring any changes in immune disposition of the graft recipient toward graft alloantigens. However, such changes can be revealed by challenge with donor alloantigens at a distant site (DTH challenge). In allograft acceptor mice, this disposition evolves from pro-inflammatory to anti-inflammatory.

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=> s l11 and IL15
L13           0 L11 AND IL15

=> s l1 and "IL-15"
L14          43 L1 AND "IL-15"

=> s l14 and "IL-2"
L15          26 L14 AND "IL-2"

=> s l15 and "TGF beta"
L16           0 L15 AND "TGF BETA"

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PROCESSING COMPLETED FOR L15
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L17 16 DUP REMOVE L15 (10 DUPLICATES REMOVED)

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L18 0 L17 AND "EX VIVO"

=> d l17 1-16 cbib abs

L17 ANSWER 1 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:343373 Document No.: PREV200200343373. Involvement of **IL-15** in indirect presentation: Implications for chronic rejection. Batten, P. (1); McCormack, A. M. (1); Gracie, J. A.; Yacoub, M. H. (1); Rose, M. L. (1). (1) National Heart and Lung Institute at Harefield Hospital, Imperial College School of Science, Technology and Medicine, Harefield, Middlesex UK. Journal of Heart and Lung Transplantation, (January, 2002) Vol. 21, No. 1, pp. 71. http://www.elsevier.com/locate/hea lun. print. Meeting Info.: Twenty-Second Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplantation Washington, DC, USA April 10-13, 2002 International Society for Heart and Lung Transplantation. ISSN: 1053-2498. Language: English.

L17 ANSWER 2 OF 16 MEDLINE
2001523528 Document Number: 21455855. PubMed ID: 11571459. Selective inhibition of **IL-2** gene expression by **IL-2** antisense oligonucleotides blocks heart allograft rejection. Qu X; Kirken R A; Tian L; Wang M; Bennett C F; Stepkowski S M. (Division of Immunology and Organ Transplantation, Department of Surgery, The University of Texas Medical School-Houston, 6431 Fannin, Suite 6.240, Houston, TX 77030, USA.) TRANSPLANTATION, (2001 Sep 15) 72 (5) 915-23. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB PURPOSE: We tested the effects of selective inhibition of interleukin (**IL**)-**2** gene expression by **IL-2** antisense oligonucleotide (oligo) with phosphorothioate (PS)/phosphodiester (PD)/2'-methoxyethyl (ME) modifications (17359) on T-cell function and the survival of heart allografts in mice. **METHODS**: The PS- (17328) or PS/PD/ME- (17359) **IL-2** oligo was electroporated to mouse T cell lymphoma cells (TIB 155) stimulated with concanavalin A (Con A). Expression of **IL-2** was analyzed by an ELISA spot assay and a reverse transcript polymerase chain reaction **method**. C3H (H-2k) mice transplanted with BALB/c (H-2d) heart grafts were treated i.v. with a 7-day osmotic pump with 20 mg/kg 17359 alone or in combination with sirolimus (SRL). RESULTS: In comparison with untreated controls, 500 to 2000 nM 17328 inhibited **IL-2** protein production by 21.8% to 47.2%, whereas 500 to 2000 nM 17359 did so by 35.5% to 83.5% (both P<0.001). In vivo, 20 mg/kg 17359 prolonged survivals to a mean survival time (MST) of 18.3+/-2.6 days (P<0.001) in comparison with only 8.2+/-0.8 days in untreated controls. Although 0.2 mg/kg SRL alone produced a MST of 18.8+/-6.0 days (P<0.01), addition of 20 mg/kg 17539 synergistically extended survivals to 54.3+/-12.1 days (P<0.001). As expected, **IL-2** mRNA, but not IL-7, IL-9, or **IL-15** mRNA, was reduced in allografts from recipients treated with 17359 compared with untreated controls. Lymph node cells from the same recipients displayed reduction in proliferative response to donor alloantigen and in generation of alloantigen-specific cytotoxic T cells. CONCLUSION: Selective inhibition of **IL-2** mRNA in vivo inhibits T-cell function and extends allograft survival.

L17 ANSWER 3 OF 16 MEDLINE DUPLICATE 1
2001523524 Document Number: 21455851. PubMed ID: 11571455. Interleukin-15 is the main mediator of lymphocyte proliferation in cultures mixed with human kidney tubular epithelial cells. Lewis E; Weiler M; Chaimovitz C; Douvdevani A. (Department of Nephrology, Soroka University Medical Center

and Ben-Gurion University of the Negev, PO Box 151, Beer-Sheva 84101, Israel.) TRANSPLANTATION, (2001 Sep 15) 72 (5) 886-90. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Interleukin- (**IL**) **15** is a potent non-T cell-derived cytokine with **IL-2**-like activities. Elevated levels of **IL-15** expression were observed in biopsies of acutely rejected human kidney grafts. We tested the role of **IL-15** in mixed lymphocyte kidney reaction (MLKR) and the effects of immunosuppressive drugs on this reaction. **METHODS:** Primary cultures of human tubular epithelial cells (TEC) were stimulated by interferon-gamma and treated with cyclosporin A (CsA, 10-1000 ng/ml), rapamycin (Rapa, 2.5-10 ng/ml), and dexamethasone (Dex, 10-10-10-7 M). IL-15 **levels were** measured by ELISA. To induce MLKR, we seeded OKT3-prestimulated allogenic human peripheral blood mononuclear cells (PBMC) on a monolayer of TEC in the presence of CsA (25-250 ng/ml), Rapa (0.25-1 ng/ml), and Dex (10-10-10-7 M). PBMC proliferation was quantified by 3H-thymidine incorporation. RESULTS: CsA, Dex, and Rapa had no effect on IL-15 production by **TEC. The** presence of TEC induced marked proliferation of PBMC. Pretreatment of TEC with IFN-gamma enhanced MLKR in direct correlation with the increased IL-15 levels. MLKR **was blocked** by anti-IL-15, but not **significantly by** anti-IL-2 monoclonal antibody. **Contrary to** Rapa and Dex, CsA failed to inhibit MLKR proliferation in MLKR. Its production by TEC was unaffected by CsA, Rapa, and Dex. However, IL-15 activity is **effectively inhibited** by Rapa and Dex but not by CsA. The diversity in the effects of the various drugs is probably related to the different mechanisms. Our results support the possible involvement of renal IL-15 in graft **rejection and** suggest **that resistance** to CsA treatment is related to its failure to decrease IL-15 activity.

L17 ANSWER 4 OF 16 MEDLINE
2001205811 Document Number: 21137926. PubMed ID: 11239443. Contrasting roles of **IL-2** and **IL-15** in the life and death of lymphocytes: implications for immunotherapy. Waldmann T A; Dubois S; Tagaya Y. (Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.. tawald@helix.nih.gov) . IMMUNITY, (2001 Feb) 14 (2) 105-10. Ref: 52. Journal code: 9432918. ISSN: 1074-7613. Pub. country: United States. Language: English.

L17 ANSWER 5 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:541106 Document No.: PREV200100541106. Neither NK cells nor host innate immune signaling through the common cytokine receptor gamma chain are required for islet xenograft rejection. Johnson, Z. A.; Gill, R. G.. Xenotransplantation, (August, 2001) Vol. 8, No. Supplement 1, pp. 9. print. Meeting Info.: VI Congress of the International Xenotransplantation Association Chicago, Illinois, USA September 29-October 03, 2001 ISSN: 0908-665X. Language: English. Summary Language: English.

L17 ANSWER 6 OF 16 MEDLINE
2000483262 Document Number: 20432333. PubMed ID: 10975865. Selective blockade of **IL-15** by soluble **IL-15** receptor alpha-chain enhances cardiac allograft survival. Smith X G; Bolton E M; Ruchatz H; Wei X; Liew F Y; Bradley J A. (Department of Surgery, University of Cambridge, Cambridge, United Kingdom.) JOURNAL OF IMMUNOLOGY, (2000 Sep 15) 165 (6) 3444-50. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB **IL-15** is a T cell growth factor that shares many functional similarities with **IL-2** and has recently been shown to be present in tissue and organ allografts, leading to

speculation that **IL-15** may contribute to **graft rejection**. Here, we report on the in vivo use of an **IL-15** antagonist, a soluble fragment of the murine IL-15R alpha-chain, to investigate the contribution of **IL-15** to the rejection of fully vascularized cardiac allografts in a mouse experimental model. Administration of soluble fragment of the murine IL-15R alpha-chain (sIL-15Ralpha) to CBA/Ca (H-2k) recipients for 10 days completely prevented rejection of minor histocompatibility complex-mismatched B10.BR (H-2k) heart grafts (median survival time (MST) of >100 days vs MST of 10 days for control recipients) and led to a state of donor-specific immunologic tolerance. Treatment of CBA/Ca recipients with sIL-15Ralpha alone had only a modest effect on the survival of fully MHC-mismatched BALB/c (H-2d) heart grafts. However, administration of sIL-15Ralpha together with a single dose of a nondepleting anti-CD4 mAb (YTS 177.9) delayed mononuclear cell infiltration of the grafts and markedly prolonged graft survival (MST of 60 days vs MST of 20 days for treatment with anti-CD4 alone). Prolonged graft survival was accompanied in vitro by reduced proliferation and IFN-gamma production by spleen cells, whereas CTL and alloantibody levels were similar to those in animals given anti-CD4 mAb alone. These findings demonstrate that **IL-15** plays an important role in the rejection of a vascularized organ allograft and that antagonists to **IL-15** may be of therapeutic value in preventing allograft rejection.

DUPLICATE 2

L17 ANSWER 7 OF 16 MEDLINE PubMed ID: 11045645. Cytokine and
2000500267 Document Number: 20498344.
cytotoxic molecule gene expression determined in peripheral blood mononuclear cells in the diagnosis of acute renal rejection. Dugre F J; Gaudreau S; Belles-Isles M; Houde I; Roy R. (Centre de recherche et Immunologie, CHUQ, Universite Laval, Quebec, Canada.) TRANSPLANTATION, (2000 Oct 15) 70 (7) 1074-80. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Prevention of acute rejection is the most prevalent measure used to reduce the long-term risk of chronic allograft rejection. Until now, biopsy was the only useful diagnostic tool for monitoring allograft acute rejection, but invasiveness of this procedure limits its use. The aim of this study was to investigate the implication of peripheral blood immune markers as a predictive diagnostic tool preceding biopsy in acute renal allograft rejection determination. **METHODS:** Of the 61 patients studied, 13 had no rejection episodes, 8 had a proven acute rejection, and 40 were excluded for graft dysfunction causes. Mitogen-induced peripheral blood mononuclear cells were tested for interleukin- (**IL**) **2**, IL-4, IL-5, IL-6, IL-10, **IL-15**, Interferon-gamma, Perforin, Granzyme B, and Fas L using semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). An up-regulated mRNA expression value was calculated in which a patient's sample was deemed positive if its differential expression value was equal or higher than the mean differential expression value calculated from the nonrejecting patients. RESULTS: IL-4, IL-5, IL-6, Interferon-gamma, Perforin, and Granzyme B mRNA levels were associated with acute rejection. When at least two of these cytokine markers were up-regulated in a given patient, 75% of the rejecting recipients were identified against 15% of the nonrejecting patients. CONCLUSIONS: We have shown that acute rejection episodes in renal transplant recipients were associated with an increase in mRNA expression of cytokines in mitogen-induced peripheral blood mononuclear cells. The evaluation of pro-inflammatory cytokines and cytotoxic molecules prove useful in the clinical identification of acutely rejecting transplant recipients and in the justification of concomitant antirejection therapy before histological diagnosis confirmation.

DUPLICATE 3

L17 ANSWER 8 OF 16 MEDLINE PubMed ID: 10708100. Prevention of
2000170373 Document Number: 20170373.

acute allograft rejection in nonhuman primate lung transplant recipients: induction with chimeric anti-interleukin-2 receptor monoclonal antibody improves the tolerability and potentiates the immunosuppressive activity of a regimen using low doses of both microemulsion cyclosporine and 40-O-(2-hydroxyethyl)-rapamycin. Hausen B; Gummert J; Berry G J; Christians U; Serkova N; Ikonen T; Hook L; Legay F; Schuler W; Schreier M H; Morris R E. (Transplantation Immunology, Department of Cardiothoracic Surgery, Stanford University, Palo Alto, California 94305, USA.. hausen@leland.stanford.edu) . TRANSPLANTATION, (2000 Feb 27) 69 (4) 488-96. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: In previous studies of cynomolgus monkey lung allograft recipients, we demonstrated significant immunosuppressive efficacy but reduced tolerability after combined treatment with high doses of microemulsion cyclosporine (CsA) and SDZ RAD (40-O-(2-hydroxyethyl)-rapamycin). The current study was designed to compare efficacy and tolerability of a combination of low-dose CsA and high-dose SDZ RAD (CTL group) to triple therapy using the chimeric anti-interleukin-2 (**IL-2**) receptor (CD25) monoclonal antibody (mAb) basiliximab (anti-**IL-2** receptor mAb) for induction therapy (basiliximab: 5 mg intravenously on days 0 and 4) plus low-dose CsA and low-dose SDZ RAD for maintenance immunosuppression (CD25 group). CsA and anti-**IL-2** receptor mAb are drugs that reduce cytokine synthesis and block **IL-2**-mediated lymphocyte stimulation, respectively. SDZ RAD blocks lymphocyte stimulation by other cytokines (e.g., **IL-15**) that are not inhibited by anti-**IL-2** receptor mAb. **METHODS**: Twelve unilateral lung transplants were performed. Recipients were observed for 49 days by daily weight assessment, hemograms, blood chemistries, radiographs, and lung biopsies. Monkeys were euthanized before day 49 in the event of excessive weight loss (>25%) or organ failure. Target CsA trough levels were 100-200 ng/ml. Target SDZ RAD trough levels in the CTL group (no mAb) were 20-40 ng/ml, and 10-20 ng/ml in the CD25 group. RESULTS: None of the monkeys in the CD25 group needed to be euthanized early due to signs of drug toxicity. In contrast, four monkeys in the CTL group were sacrificed on days 28-35 as a result of excessive weight loss (n=3) and renal functional impairment (n=1). Three recipients in the CD25 group were euthanized on days 36, 38, and 46 as a result of persistent high fever associated with severe rejection. The median animal survival in the CTL group was 32 vs. 46 days in the CD25 group (P<0.04). The only two long-term survivors in the CTL group showed moderate rejection at day 49. The median rejection scores at day 14 (A0) and day 28 (A2) were identical in the two groups, despite the fact that the mean SDZ RAD trough level was significantly lower in the CD25 group (CTL: 38+/-3 ng/ml, CD25: 18+/-2 ng/ml, P<0.0001). After basiliximab levels fell below the minimum therapeutic level (1 mg/ml) on day 28, the median rejection score at day 49 increased to A4 in the CD25 group. CONCLUSION: This is the first study to combine an anti-**IL-2** receptor mAb with a drug from the rapamycin class plus CsA. Our study shows that induction therapy with basiliximab enabled SDZ RAD blood levels to be significantly reduced, which led to improved tolerability without the penalty of increased rejection.

DUPLICATE 4

L17 ANSWER 9 OF 16 MEDLINE

2000168555 Document Number: 20168555. PubMed ID: 10706038. Functional responses of T cells blocked by anti-CD25 antibody therapy during cardiac rejection. Baan C C; van Gelder T; Balk A H; Knoop C J; Holweg C T; Maat L P; Weimar W. (Department of Internal Medicine I, University Hospital Rotterdam-Dijkzigt, The Netherlands.. baan@inw1.azr.nl) . TRANSPLANTATION, (2000 Feb 15) 69 (3) 331-6. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Despite anti-CD25 (interleukin [**IL**]-**2** receptor alpha chain) monoclonal antibody (mAb) therapy, rejection can still occur. T-cell activation through the **IL-2**

receptor beta and gamma chains by **IL-2** or other growth factors may contribute to this rejection. Recently, we have demonstrated that the T-cell growth factor **IL-15** was abundantly present in rejecting cardiac grafts during anti-CD25 mAb treatment. **METHODS:** To test whether **IL-2**- and **IL-15**-responsive T cells play an active role in rejection during anti-CD25 mAb therapy, we measured the frequency of **IL-2**- and **IL-15**-proliferative T cells in peripheral blood from treated patients during rejection (n=12). Measurements were made by limiting dilution analysis in the absence and presence of extra in vitro-added mouse anti-human CD25 mAb. RESULTS: In the absence of anti-CD25 mAb, the frequencies of peripheral T cells responding to recombinant human (rh)**IL-2** and rhIL-15 from patients were lower than those measured in samples of healthy controls (n=7): median of **IL-2**-responding T cells 78 per 10(6) (range 31-210 per 10(6)) vs. 154 per 10(6) (122-484 per 10(6), P=0.008) and median of **IL-15**-responding T cells 62 per 10(6) (range 19-207 per 10(6)) vs. 129 per 10(6) (range 79-192 per 10(6), P=0.02), respectively. In the presence of extra in vitro-added anti-CD25 mAb, frequencies of **IL-2**-responding T cells from patients significantly decreased, although a considerable number of T cells still proliferated on rhIL-2 (median 85%, range 46-100%). In contrast, the frequencies of **IL-15** T cells still responding remained stable (median 2%, range 0-50%, P<0.001). CONCLUSIONS: Treatment with anti-CD25 mAbs cannot provide complete suppression of T-cell function because significant numbers of **IL-2**- and **IL-15**-responsive T cells remain present in the peripheral blood of allograft recipients during anti-CD25 mAb treatment.

DUPLICATE 5

L17 ANSWER 10 OF 16 MEDLINE

1999213828 Document Number: 99213828. PubMed ID: 10199736. Anti-CD25 therapy reveals the redundancy of the intragraft cytokine network after clinical heart transplantation. Baan C C; Knoop C J; van Gelder T; Holweg C T; Niesters H G; Smeets T J; van der Ham F; Zondervan P E; Maat L P; Balk A H; Weimar W. (Department of Internal Medicine I, University Hospital Rotterdam-Dijkzigt, The Netherlands.. baan@inw1.azr.nl) . TRANSPLANTATION, (1999 Mar 27) 67 (6) 870-6. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Despite blockade of the interleukin-2/interleukin 2 receptor (**IL-2**/IL-2R) pathway by the murine anti-CD25 (i.e., IL-2R alpha chain) monoclonal antibody BT563, cardiac rejection can still occur. In these cases, growth factors other than **IL-2** may contribute to allograft rejection. We studied the expression of **IL-15**, a macrophage-derived cytokine associated with T-cell activation, which interacts with the beta and gamma chains of the IL-2R during rejection episodes under anti-CD25 therapy. **METHODS:** We measured intragraft **IL-15** mRNA expression and the number of **IL-15**- and CD68-positive cells in posttransplantation endomyocardial biopsies (EMBs; n=45) and in nontransplanted, donor-heart specimens (n=11) by competitive template reverse transcription-polymerase chain reaction and immunohistochemistry, respectively. RESULTS: **IL-15** mRNA expression was present in the majority of posttransplantation EMB specimens (91%, 41/45) and in nontransplanted donor-heart specimens (91%, 10/11). Relative **IL-15** mRNA levels were neither associated with transplantation nor with rejection status. After transplantation, the number of **IL-15**- and CD68-positive cells significantly increased (P<0.001), but **IL-15**-positive cell counts did not reflect the histological rejection grade. Anti-CD25 treatment, in contrast to its effects on the **IL-2**/IL-2R complex, had no influence on intragraft **IL-15** mRNA and protein production. In rejection EMB specimens, during (n=5) and after (n=8) anti-CD25 therapy, no differences in relative **IL-15**

mRNA levels, or in **IL-15**- and CD68-positive cell counts, were measured. CONCLUSIONS: After heart transplantation, high numbers of **IL-15**- and CD68-positive cells infiltrate the graft. This phenomenon is independent of the rejection status. **IL-15** remains present during blockade of the **IL-2**/IL-2R pathway by anti-CD25 monoclonal antibodies, and it may participate in T cell-dependent donor-directed immune responses, thereby explaining the occurrence of rejection in the absence of **IL-2**.

L17 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:498410 Document No.: PREV199900498410. Graft-infiltrating cells synthesize more sTNFRI pre- and during acute rejection. Oliveira, J. G. G. (1); Xavier, P.; Sampaio, S. (1); Mendes, A. A.; Guerra, L. E. R. (1). (1) Depart. of Nephrol, H.S.J, Porto Portugal. Journal of the American Society of Nephrology, (Sept., 1999) Vol. 10, No. PROGRAM AND ABSTR. ISSUE, pp. 709A-710A. Meeting Info.: 32nd Annual Meeting of the American Society of Nephrology Miami Beach, Florida, USA November 1-8, 1999 American Society of Nephrology. ISSN: 1046-6673. Language: English.

DUPLICATE 6

L17 ANSWER 12 OF 16 MEDLINE
1999098727 Document Number: 99098727. PubMed ID: 9884258. Immune-activation gene expression in clinically stable renal allograft biopsies: molecular evidence for subclinical rejection. Lipman M L; Shen Y; Jeffery J R; Gough J; McKenna R M; Grimm P C; Rush D N. (Department of Medicine and Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, Canada.. mdip@musica.mcgill.ca) . TRANSPLANTATION, (1998 Dec 27) 66 (12) 1673-81. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: A significant percentage of biopsies from stable, well-functioning renal allografts have histologic findings consistent with acute rejection or borderline rejection. The implication of this finding is not yet fully understood. We analyzed immune-activation gene transcripts in stable protocol biopsies to determine the extent of immunologic activity of graft-infiltrating cells in this setting. Histologic classification of the biopsies was based on the Banff criteria. To emphasize that the tissue samples were procured from grafts with no clinical evidence of impaired function, we interjected the term "subclinical" into the Banff terminology. This produced three histologic categories: normal, borderline subclinical rejection, and acute subclinical rejection. **METHODS**: We used competitive template polymerase chain reaction techniques to quantify transcript amounts for the constant region of the T-cell receptor beta chain; the cytokines, tumor necrosis factor alpha, interleukin (IL)-1beta, transforming growth factor beta, interferon gamma, **IL-2**, IL-4, IL-10, and **IL-15**; and the cytotoxic T lymphocyte effector molecules, granzyme B, perforin, and Fas ligand. RESULTS: We found that histologically normal biopsies were typically devoid of gene transcripts or had very low amounts. Conversely, biopsies with acute subclinical rejection by histologic examination had heightened amounts of transcripts for many of the genes assayed. Borderline subclinical rejection samples showed an intermediate amount of expression. CONCLUSIONS: These results demonstrate that histologic features of rejection are often accompanied by enhanced expression of pro-inflammatory gene transcripts, despite the absence of clinically overt graft dysfunction. As this state of subclinical rejection could prove detrimental to long-term graft function, a role for surveillance biopsies of stable grafts with intent to treat subclinical rejection should be considered.

L17 ANSWER 13 OF 16 MEDLINE
1998143290 Document Number: 98143290. PubMed ID: 9484761. Blockade of the interleukin (**IL**)-**2**/**IL-2** receptor

pathway with a monoclonal anti-**IL-2** receptor antibody (BT563) does not prevent the development of acute heart allograft rejection in humans. van Gelder T; Baan C C; Balk A H; Knoop C J; Holweg C T; van der Meer P; Mochtar B; Zondervan P E; Niesters H G; Weimar W. (Department of Internal Medicine, University Hospital Rotterdam Dijkzigt, The Netherlands.) TRANSPLANTATION, (1998 Feb 15) 65 (3) 405-10. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Anti-interleukin (**IL**)-**2** receptor (IL-2R) antibodies have been used as rejection prophylaxis after organ transplantation. Despite this induction treatment, acute rejections may occur. We wondered whether these rejections developed via the **IL-2**/IL-2R pathway. **METHODS:** In a prospective trial using BT563, a murine IgG1 anti-IL-2R antibody, for rejection prophylaxis after heart transplantation, 20 patients were treated in combination with cyclosporine from the day of transplantation (group A). As a control group, 31 patients were also treated with BT563, but in these patients, cyclosporine treatment was initiated on day 3 (group B). RESULTS: Three patients from group A and two patients from group B died in the first postoperative month (of causes not related to acute rejection) and were left out of the analysis of rejection incidence. Freedom from acute rejection at 1 week after transplantation in group A (14/17; 82%) was lower than in group B (16/29; 55%), although the difference did not reach statistical significance. There was no difference in either the number of acute rejection episodes at 12 weeks or the required rejection treatments between groups A and B. Infectious complications were evenly distributed in both groups. Immunohistochemistry showed that during acute rejection, in the presence of circulating BT563, IL-2R-bearing cells were present in only one of five rejection biopsies (20%), whereas these cells were often present (6/8, or 75%) in rejections occurring in the absence of BT563. The presence of BT563 was associated with a similar difference in the mRNA expression of **IL-2** (2/5 vs. 6/8). CONCLUSIONS: Apparently, despite adequate blockade of the **IL-2**/IL-2R pathway, patients may develop acute rejection, reflecting the redundancy of the cytokine network. The ever-present **IL-15** may well be a candidate for overtaking the role of **IL-2**.

DUPLICATE 7

L17 ANSWER 14 OF 16 MEDLINE

1998365006 Document Number: 98365006. PubMed ID: 9701276. Differential expression of T-cell growth factors in rejecting murine islet and human renal allografts: conspicuous absence of interleukin (IL)-9 despite expression of **IL-2**, IL-4, IL-7, and **IL-15**. Li X C; Schachter A D; Zand M S; Li Y; Zheng X X; Harmon W E; Strom T B. (Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, Children's Hospital, Boston, Massachusetts 02215, USA.) TRANSPLANTATION, (1998 Jul 27) 66 (2) 265-8. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Interleukin (**IL**)-**2**, IL-4, IL-7, IL-9, and **IL-15**, all T-cell growth factors (TCGFs), utilize the common **IL-2** receptor gammac chain as a critical signaling component in their receptor complexes. We have bred **IL-2**-/- and IL-4-/- double knockout (DKO) mice and showed vigorous islet allograft rejection by DKO hosts. The identity of TCGFs that support the **IL-2**- and IL-4-independent allograft rejection is unclear. **METHODS:** We analyzed IL-9 gene expression in rejecting islet allografts in wild-type and in DKO mice, as well as in human renal transplant biopsy specimens, by reverse transcriptase polymerase chain reaction and compared the expression of IL-9 with that of other TCGFs. RESULTS: IL-9 gene expression was not detected in rejecting murine islet allografts in either wild-type or DKO recipient mice despite robust expression of other TCGFs, including IL-7 and **IL-15**. IL-9 transcripts were also not expressed in any of the human renal

transplant biopsies obtained 4 to 251 days after transplantation, regardless of the presence or absence of histological evidence of rejection. Despite expression of IL-9 by DKO splenic cells upon in vitro mitogenic stimulation, IL-9 alone was unable to stimulate the proliferation of concanavalin A-activated splenic leukocytes harvested from DKO mice. CONCLUSION: IL-9 is conspicuously absent despite vigorous expression of **IL-2**, IL-4, IL-7, and **IL-15** genes during acute allograft rejection.

L17 ANSWER 15 OF 16 MEDLINE
1998306480 Document Number: 98306480. PubMed ID: 9642512. Increased intragraft **IL-15** mRNA expression after liver transplantation. Baan C C; Niesters H G; Metselaar H J; Mol W M; Loonen E H; Zondervan P E; Tilanus H W; IJzermans J M; Schalm S W; Weimar W. (Department of Internal Medicine, University Hospital Rotterdam-Dijkzigt, The Netherlands.. baan@inwl.azr.nl) . CLINICAL TRANSPLANTATION, (1998 Jun) 12 (3) 212-8. Journal code: 8710240. ISSN: 0902-0063. Pub. country: Denmark. Language: English.

AB To study T-cell/macrophage interactions at the molecular level in clinical allograft rejection, we measured intragraft mRNA expression of the T-cell derived cytokine **IL-2** and the macrophage derived chemokine **IL-15**, a novel cytokine associated with T-cell activation, in post-transplant liver biopsies (n = 33) and in non-transplanted control liver tissue by reverse transcriptase-polymerase chain reaction (RT-PCR). We analyzed biopsies without evidence of rejection (n = 12), with spontaneously resolving histological rejection (n = 10), or with histological rejection accompanied with clinical rejection (n = 11) defined by rising serum bilirubin and aspartate amino transaminase levels. **IL-15** mRNA expression was present in the majority of post-transplant liver biopsies (91%, 30/33) and was significantly upregulated as compared with non-transplanted liver tissue (p = 0.005). However, the increased intragraft **IL-15** mRNA level was not indicative for rejection. In contrast to intragraft **IL-15** mRNA expression, **IL-2** mRNA transcription was measured in the minority of the post-transplant liver biopsies (15%, 5/33) and not detectable in control specimens. In addition, **IL-2** mRNA was almost specifically measured in rejection biopsies concurrent with graft dysfunction (36%, 4/11 versus 1/22 without clinical rejection; p = 0.03). No relation between intragraft **IL-2** and **IL-15** mRNA expression was found. The **IL-15** mRNA expression levels were not higher in the **IL-2** negative rejections compared with those in **IL-2** positive rejections. To conclude, in contrast to **IL-2**, the function of **IL-15** in T-cell mediated rejection remains unclear. The overall high **IL-15** mRNA levels in sites of immune responses suggests that the macrophage-derived mediator **IL-15** is involved in a constant flow of T-cells from the circulation into the graft.

L17 ANSWER 16 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:413059 Document No.: PREV199699135415. The intragraft gene activation of markers reflecting T-cell-activation and -cytotoxicity analyzed by quantitative RT-PCR in renal transplantation. Strehlau, J.; Pavlakis, M.; Lipman, M.; Maslinski, W.; Shapiro, M.; Strom, T. B. (1). (1) Dep. Med., Div. Immunol., Harv. Med. Sch., 330 Brookline Ave., Boston, MA 02215 USA. Clinical Nephrology, (1996) Vol. 46, No. 1, pp. 30-33. ISSN: 0301-0430. Language: English.

AB T-cell activation is the key event in the development of acute allograft rejection and precedes clinically apparent organ damage. We have performed competitive RT-PCR to quantify the intragraft gene expression for T-cell associated cytokines (**IL-2**, IL-4, IL-7, **IL-15**), CTLA4 and cytotoxic lymphocyte specific molecules to test their potential as rejection markers and to further elucidate mechanisms

involved in **graft rejection**. RNA was isolated from snap-frozen portions of core biopsies obtained for the evaluation of graft dysfunction in 34 adults and 8 children. Reverse transcription derived cDNA was coamplified with a known amount of a competitor (a mutated target gene fragment) and normalized for the house keeping gene GAPDH. **IL -2**, the principal T-cell growth factor and IL-4 were not detectable in any biopsy at the time of histologically apparent rejection. Transcripts of the novel cytokine **IL-15** were found in all dysfunctioning grafts and in two donor kidneys prior to reperfusion. CTLA-4, expressed in activated T-cells after costimulation by CD28 was uniformly present post transplantation, but not in the two donor kidneys. Transcripts for IL-7 (p lt 0.001), **IL-15** (p lt 0.0005), CTLA4 (p=0.04), granzyme B (p lt 0.00015) and perforin (p lt 0.0003) showed a significant correlation to acute rejection episodes. Heightened gene expression declined rapidly after initiation of rejection treatment. Fas-ligand mRNA gene expression was upregulated in both acute and chronic rejections. While this study shows that competitive RT-PCR is a reliable diagnostic tool to detect acute rejection in renal core biopsies, a future challenge will be to identify molecular markers of evolving rejections utilizing RT-PCR in sequential samples of fine needle aspirations, urine and blood.

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Selective removal of alloreactive cells from haematopoietic stem cell grafts: graft engineering for GVHD prophylaxis.

Koh MB, Prentice HG, Lowdell MW.

Department of Haematology, Royal Free Campus, Royal Free and University College Medical School, London, UK.

One of the main goals in allogeneic bone marrow transplantation is the abrogation of graft-versus-host disease with the preservation of antileukaemia and antiviral activity. We have established a novel system for the selective removal of alloreactive lymphocytes from donor grafts while retaining an effective allogeneic response to third-party stimulator cells. Initial feasibility studies were done with unrelated HLA-mismatched pairs and then extended into the matched setting. Mononuclear cells from HLA-matched donors were cocultured with irradiated recipient cells prestimulated with cytokines (gamma-IFN and TNF-alpha) in a modified mixed lymphocyte culture (MLC). Alloreactive donor lymphocytes were identified by expression of CD69, an early activation marker and selectively removed by paramagnetic bead sorting. The remaining 'non-alloreactive' lymphocytes were tested in proliferative assays against the original matched recipient and to a third-party donor. A mean depletion of proliferative capacity to 11.5 +/- 9.9% of the original matched recipient response was achieved while the residual third-party response was largely preserved at 77.8 +/- 20.9% which should translate into improved immune reconstitution and preservation of antiviral activity. The non-alloreactive lymphocytes could also possess functional antileukaemia activity. Moreover, the alloreactive cells are easily recoverable in this selective T cell depletion strategy for cryopreservation and ready for immediate access as therapeutic donor lymphocyte infusions in cases of frank relapse post transplant.

PMID: 10373075 [PubMed - indexed for MEDLINE]

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Contribution of CD4+ and CD8+ T cells to graft-versus-host disease and graft-versus-leukemia reactivity after transplantation of MHC-compatible bone marrow.

Truitt RL, Atasoylu AA.

Department of Pediatrics, Medical College of Wisconsin, Milwaukee 53226.

A murine model of allogeneic bone marrow (BM) transplantation was used to determine the relative importance of CD4+ and CD8+ T cells in establishing donor T cell chimerism and in the development of graft-versus-host (GVH) and graft-versus-leukemia (GVL) reactivity. Mature donor T cells were essential for complete chimerism when host mice (AKR, H-2k) were conditioned with suboptimal irradiation (9 Gy = LD50). Transplantation of donor BM (B10.BR, H-2k) resulted in mixed chimerism, whereas mice given BM containing additional T cells developed into complete and stable chimeras. Depletion of T cell subsets was associated with an increase in the frequency of mixed chimerism. The incidence of lethal GVHD was dependent on the number of T cells added to the BM inoculum. Ex vivo depletion of CD4+ T cells eliminated GVH-associated mortality. Removal of CD8+ T cells had no effect on overall survival. In contrast to the GVH results, removal of either CD4+ or CD8+ T cells compromised GVL reactivity, indicating that an optimal GVL response required both CD4+ and CD8+ T cells. T cell-subset depletion did not interfere with the induction of donor-host tolerance in these chimeras and may have facilitated its development. The loss of GVH/GVL effector cells as a result of T cell depletion and the development of donor-host tolerance may act synergistically to prevent or suppress GVH and GVL reactivity.

PMID: 1833016 [PubMed - indexed for MEDLINE]

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1: Immunology 1997 Mar;90(3):397-401 Related Articles, Books, LinkOut

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Cord blood CD4+ CD45RA+ T cells achieve a lower magnitude of activation when compared with their adult counterparts.

Hassan J, Reen DJ.

Our Lady's Hospital for Sick Children, Dublin, Ireland

Highly purified CD4+ CD45RA+ cells from cord blood and peripheral blood from healthy adults were studied. The levels of expression of the CD2, CD3, CD4 and CD28 antigens were similar; however, CD45 and CD45RA antigen expression were slightly lower in cord cells. The reduced expression of the CD45RA antigen on cord CD4+ T cells was confirmed in whole blood. Functional assessment revealed deficiencies in cord CD4+ CD45RA+ T cells. Interleukin-2 (IL-2) production in response to specific triggering via CD2 monoclonal antibody (mAb) alone, or CD2 mAb in combination with CD28 mAb showed marked underproduction (about 10% of adult production). When CD25 expression was examined, it was observed that the proportion of activated CD4+ CD45RA+ T cells in cord blood was lower than in adult (about 20% of adult expression). Proliferation to CD2 mAbs or CD2 + 28 mAbs of cord blood native cells was similarly depressed. Investigation of IL-2 mRNA expression under these stimulatory conditions paralleled the results observed for CD25 expression, IL-2 production and proliferation. When phorbol 12-myristate 13-acetate (PMA) was added to the cells triggered with CD2 + 28mAbs, the responses examined were enhanced in both cord and adult blood with no significant differences between the groups. These findings suggest that under identical conditions of stimulation, purified cord blood CD4+ CD45RA+ T cells do not acquire similar activation status as their adult counterparts. These findings may help in understanding the reduced graft-versus-host disease (GVHD) observed in cord blood stem cell transplantation.

PMID: 9155647 [PubMed - indexed for MEDLINE]

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Differential effect of transforming growth factor-beta 1 on the activation of human naive and memory CD4+ T lymphocytes.

de Jong R, van Lier RA, Ruscetti FW, Schmitt C, Debre P, Mossalayi MD.

Laboratoire d'Immunologie Cellulaire et Tissulaire, CNRS URA 625, CHU Pitie-Salpetriere, Paris, France.

Transforming growth factor-beta 1 (TGF-beta 1) can have stimulatory or inhibitory effects on cell growth. For several cell types, the effect of TGF-beta 1 was found to correlate with the differentiation stage of the cells and the presence of other cytokines. We have studied here the influence of TGF-beta 1 on CD4+ T cell activation in relation to the differentiation stage of the cells by evaluating the effect of TGF-beta 1 on the proliferative responses of purified CD4+CD45RA+ (unprimed) and CD4+CD45RO+ (primed) lymphocytes. Under certain conditions, TGF-beta 1 exerted a co-stimulatory effect on peripheral blood CD4+CD45RA+ T cells whereas the outgrowth of CD4+CD45RO+ T cells was suppressed in any activation system tested. The enhancement of proliferative responses by TGF-beta 1 in TCR/CD3 or CD2 stimulated cultures of CD45RA+ cells involved up-regulation of CD25 expression and was dependent on the presence of exogenous IL-2 or CD28 mAbs; IL-7 driven proliferative responses were suppressed by TGF-beta 1. These observations were confirmed in experiments with purified cord blood (CB) CD4+ T cells inasmuch as addition of TGF-beta 1 caused a 2- to 7-fold increase in IL-2 driven proliferative responses of these cells. Finally we show that, in contrast to the effect of TGF-beta 1 during primary stimulation of CD CD4+ T cells, TGF-beta 1 suppressed T cell proliferation for approximately 40% in secondary cultures of these cells.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7912547 [PubMed - indexed for MEDLINE]

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1: Exp Hematol 1999 Mar;27(3):561-8

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FULL-TEXT ARTICLE

Immune reconstitution following allogeneic peripheral blood progenitor cell transplantation: comparison of recipients of positive CD34+ selected grafts with recipients of unmanipulated grafts.

Martinez C, Urbano-Ispizua A, Rozman C, Marin P, Rovira M, Sierra J, Montfort N, Carreras E, Montserrat E.

Hematology Department, Postgraduate School of Hematology Farreras-Valenti, Institut d'Investigacions Biomediques August Pi i Sunyer, University of Barcelona, Spain.

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We compared the kinetic recovery of lymphocytes and their subsets in two groups of patients submitted to allogeneic peripheral blood progenitor cell transplantation (allo-PBT): those receiving lymphocyte-depleted leukaphereses by positive selection of CD34+ cells (group 1, n = 18) and those receiving unmanipulated leukaphereses (group 2, n = 15). Patients were conditioned with cyclophosphamide (120 mg/kg) and fractioned total body irradiation (13 Gy, group 1; 12 Gy, group 2). The mean number (x 10(6)/kg) of CD34+ and CD3+ cells infused was 4.0 and 0.67, respectively, in group 1 patients, and 4.7 and 274, respectively, for group 2 patients. Graft-versus-host disease prophylaxis consisted of cyclosporin A + methylprednisolone for group 1 and cyclosporin A + methotrexate for group 2. Median follow-up was 7 months (range 2-8 months) for both groups. During the first 6 months post-transplant, CD4+ cell counts were lower in group 1 as compared with group 2 (p = 0.014, 0.010, 0.011, 0.0003, and 0.052 at 0.5, 1, 2, 3, and 6 months, respectively), whereas there was no difference at 8 months. The number of CD4+CD45RA+ cells was very low throughout the study in both groups, being lower in group 1 than in group 2, especially during the first 3 months post-transplant (p = 0.007 and 0.0006 at 1 and 3 months). Normal levels of CD8+ cells were reached by 1 month post-transplant in both groups. TCR gamma delta + cell counts were lower in group 1 than in group 2 during the first 4 months post-transplant (p = 0.001, 0.004, and 0.04 at 1, 3, and 4 months). A normal number of natural killer cells (CD3-CD56+) was achieved 1 month post-transplant in both groups. B lymphocytes (CD19+) showed low or undetectable counts throughout the first 4 months in both groups, achieving the normal range at 8 months. These results show that, during the first 6 months following allo-PBT with CD34+ selected grafts, the number of CD4+, CD4+CD45RA+, and TCR gamma delta + cells is significantly lower than after unmanipulated allo-PBT; these

differences disappeared at 8 months. In contrast, there are no differences between transplant groups in the recovery of CD8+, CD19+, and natural killer cells.

PMID: 10089920 [PubMed - indexed for MEDLINE]

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1: Transplantation 1999 Jan 15;67(1):124-30 Related Articles, Books, LinkOut

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Effective depletion of alloreactive lymphocytes from peripheral blood mononuclear cell preparations.

Garderet L, Snell V, Przepiorka D, Schenk T, Lu JG, Marini F, Gluckman E, Andreeff M, Champlin RE.

Department of Molecular Hematology and Therapy, University of Texas M.D. Anderson Cancer Center, Houston 77030-4095, USA.

BACKGROUND: T cells present in an allogeneic bone marrow transplant may produce graft-versus-host disease but also contribute to immune reconstitution and enhance engraftment. Our aim was to separate alloreactive from nonalloreactive T lymphocytes, by performing a mixed lymphocyte culture (MLC) stimulation of donor cells, followed by selective depletion of activated cells expressing the high-affinity interleukin 2 receptor. We then characterized the resulting depleted cell fraction. METHODS: Donor peripheral blood mononuclear cells were cocultured with irradiated peripheral blood mononuclear cells from HLA-nonidentical recipient stimulators in an MLC. After 3 days, CD25+ lymphocytes (alloreactive cells expressing the alpha chain of the interleukin 2 receptor) were removed by immunomagnetic separation. The depleted donor fraction and untreated cells were then rechallenged in a secondary MLC with the original irradiated stimulator cells or a third party to assess relative alloreactivity. RESULTS: Inhibition of the secondary MLC and of host-specific cytotoxic activities was observed as well as a disappearance of interleukin 2 receptor-positive cells. Alloreactivity against unrelated third-party cells was preserved. Limiting dilution analysis of residual alloantigen-reactive T lymphocytes demonstrated a 1.3 log reduction of antihost reactivity. The depletion largely removed host-specific alloreactive CD4+ cells. CONCLUSIONS: This method reduces alloreactivity while retaining reactivity against third-party targets. This approach may allow therapeutic infusion of T cells after HLA-nonidentical allografts with a reduced capacity to produce graft-versus-host disease.

PMID: 9921808 [PubMed - indexed for MEDLINE]

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Rapid conversion of naive to effector T cell function counteracts diminished primary human newborn T cell responses.

Early E, Reen DJ.

Children's Research Centre, Our Lady's Hospital For Sick Children, Crumlin, Dublin, Ireland.

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The reduced incidence of graft versus host disease following the use of human cord blood as a source of stem cells for bone marrow reconstitution challenges our understanding of the immunocompetence of newborn T cells. Newborn CD4+ T cells express mainly the CD45RA phenotype and have been considered to respond comparably to adult CD4+ T cells exhibiting the CD45RA phenotype. We compared the in vitro kinetics of phenotypic conversion of newborn and adult CD4+CD45RA+ T cells to CD4+CD45RO+ T cells. The cytokine profile and B cell helper activity of the converted CD4+CD45RO+ T cell population were also determined. Newborn CD4+CD45RA+ T cells were converted to CD4+CD45RO+ with significantly faster time kinetics than adult CD4+CD45RA+ T cells, following either phytohaemagglutinin (PHA) or anti-CD2 activation. Freshly purified newborn naive T cells did not produce IL-2, IL-4 or interferon-gamma (IFN-gamma) following stimulation, whereas adult naive T cells secreted IL-2 and adult-derived CD4+CD45RO+ T cells secreted all three cytokines under the same stimulatory conditions. However, newborn and adult CD4+CD45RA+ T cells, following primary stimulation and maturation in vitro, acquired the ability to secrete a Th1-type cytokine profile of IL-2 and IFN-gamma after secondary stimulation. Newborn CD4+ naive T cells that acquired the CD45RO phenotype in vitro also gained B cell helper activity equivalent to that of adult in vitro matured CD4+ naive T cells. These findings suggest that newborn and adult CD4+CD45RA+ T cell subsets are differentially responsive to various stimuli. They show that newborn CD4+CD45RA+ naive T cells can transform more quickly than their adult counterparts into functionally equivalent CD4+CD45RO+ T cells, a process that may be important to counteract the immature immune environment which exists in the newborn.

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Effective depletion of alloreactive lymphocytes from peripheral blood mononuclear cell preparations.

Garderet L, Snell V, Przepiorka D, Schenk T, Lu JG, Marini F, Gluckman E, Andreeff M, Champlin RE.

Department of Molecular Hematology and Therapy, University of Texas M.D. Anderson Cancer Center, Houston 77030-4095, USA.

BACKGROUND: T cells present in an allogeneic bone marrow transplant may produce graft-versus-host disease but also contribute to immune reconstitution and enhance engraftment. Our aim was to separate alloreactive from nonalloreactive T lymphocytes, by performing a mixed lymphocyte culture (MLC) stimulation of donor cells, followed by selective depletion of activated cells expressing the high-affinity interleukin 2 receptor. We then characterized the resulting depleted cell fraction. METHODS: Donor peripheral blood mononuclear cells were cocultured with irradiated peripheral blood mononuclear cells from HLA-nonidentical recipient stimulators in an MLC. After 3 days, CD25+ lymphocytes (alloreactive cells expressing the alpha chain of the interleukin 2 receptor) were removed by immunomagnetic separation. The depleted donor fraction and untreated cells were then rechallenged in a secondary MLC with the original irradiated stimulator cells or a third party to assess relative alloreactivity. RESULTS: Inhibition of the secondary MLC and of host-specific cytotoxic activities was observed as well as a disappearance of interleukin 2 receptor-positive cells. Alloreactivity against unrelated third-party cells was preserved. Limiting dilution analysis of residual alloantigen-reactive T lymphocytes demonstrated a 1.3 log reduction of antihost reactivity. The depletion largely removed host-specific alloreactive CD4+ cells. CONCLUSIONS: This method reduces alloreactivity while retaining reactivity against third-party targets. This approach may allow therapeutic infusion of T cells after HLA-nonidentical allografts with a reduced capacity to produce graft-versus-host disease.

PMID: 9921808 [PubMed - indexed for MEDLINE]

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Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft.

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Horwitz ME, Barrett AJ, Brown MR, Carter CS, Childs R, Gallin JI, Holland SM, Linton GF, Miller JA, Leitman SF, Read EJ, Malech HL.

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BACKGROUND: The treatment of chronic granulomatous disease with conventional allogeneic hematopoietic stem-cell transplantation carries a high risk of serious complications and death. We investigated the feasibility of stem-cell transplantation without ablation of the recipient's bone marrow. METHODS: Ten patients, five children and five adults, with chronic granulomatous disease underwent peripheral-blood stem-cell transplantation from an HLA-identical sibling. We used a nonmyeloablative conditioning regimen consisting of cyclophosphamide, fludarabine, and antithymocyte globulin. The allograft was depleted of T cells to reduce the risk of severe graft-versus-host disease. Donor lymphocytes were administered at intervals of 30 days or more after the transplantation to facilitate engraftment. RESULTS: After a median follow-up of 17 months (range, 8 to 26), the proportion of donor neutrophils in the circulation in 8 of the 10 patients was 33 to 100 percent, a level that can be expected to provide normal host defense; in 6 the proportion was 100 percent. In two patients, graft rejection occurred. Acute graft-versus-host disease (grade II, III, or IV) developed in three of the four adult patients with engraftment, one of whom subsequently had chronic graft-versus-host disease. None of the five children had grade II, III, or IV acute graft-versus-host disease. During the follow-up period, four serious infections occurred among the patients who had engraftment. Three of the 10 recipients died. Preexisting granulomatous lesions resolved in the patients in whom transplantation was successful. CONCLUSIONS: Nonmyeloablative conditioning followed by a T-cell-depleted hematopoietic stem-cell allograft is a feasible option for patients with chronic granulomatous disease, recurrent life-threatening infections, and an HLA-identical family donor.

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Differential activation of CD8+ T cells by transforming growth factor-beta 1.

Lee HM, Rich S.

Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030.

Transforming growth factor-beta (TGF-beta) is a highly conserved multifunctional factor that broadly regulates cell growth and differentiation, and exhibits diverse regulatory roles in the immune system. In contrast to other studies describing TGF-beta as a potent inhibitor of lymphocyte growth, we have shown previously that TGF-beta 1 can also costimulate proliferation of murine splenic T cells activated by immobilized anti-CD3 antibody. In the present studies, we further investigate the subsets of T cells that are responsive to TGF-beta 1 costimulation. T cells were isolated into CD45RBhi/lo or CD4+/8+ populations, and their responses to TGF-beta 1 were examined. Sorted CD45RBhi cells were highly responsive to TGF-beta 1 costimulation, and proliferated to a level similar to that of unsorted T cells in response to TGF-beta 1. TGF-beta 1 also costimulated proliferation in the CD45RBlo population that was distinguished by low response and delayed kinetics. In contrast, sorted CD4+ and CD8+ T cells showed a striking differential response. Anti-CD3-stimulated proliferation of sorted CD8+ or CD4- T cells was substantially enhanced by TGF-beta 1, whereas sorted CD4+ or CD8- T cells were unresponsive. TGF-beta 1 also down-regulated CD45RB and increased CD44 expression on responsive CD8+ and CD45RBhi T cells, thereby leading to a population of T cells enriched in mature phenotype. Generation of anti-CD3-redirected lytic activity by these TGF-beta 1-costimulated CD8+ cells was strongly suppressed. However, these CD8+ T cells exhibited cytotoxic activity after restimulation in the absence of TGF-beta. TGF-beta 1 precultured CD8+ T cells also had heightened IL-2 and IFN-gamma secretion upon restimulation in comparison to cells activated initially without TGF-beta. CD8+ T cells precultured with anti-CD3 and TGF-beta remained responsive to growth enhancement by TGF-beta, although re-exposure to TGF-beta depressed other functions of these cells. Thus, TGF-beta 1 demonstrates important costimulatory roles in both growth and maturation of CD8+ T cells.

PMID: 8335902 [PubMed - indexed for MEDLINE]

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TGF-beta 1 is a potent inducer of human effector T cells.

Cerwenka A, Bevec D, Majdic O, Knapp W, Holter W.

Institute of Immunology, VIRCC at SFI, University of Vienna, Austria.

TGF-beta 1 is known to modulate lymphocyte activation affecting cell proliferation and the production of cytokines and Igs. Little is known about the characteristics of T cells grown in the presence of TGF-beta 1. We have stimulated human T cells with PHA in the presence of TGF-beta 1 under serum-free conditions for 7 days and characterized the resulting cell population. TGF-beta 1 (0.0032 to 10 ng/ml) affected neither [3H]thymidine incorporation (day 4) nor cell yield (day 7) in these cultures. However, cells activated in the presence of TGF-beta 1 proliferated vigorously in secondary cultures and produced highly elevated amounts of IL-2 (12 +/- 3-fold enhancement of IL-2 production in response to CD2 plus CD28 stimulation compared with control cells, mean +/- SEM, n = 10). The enhancing effects of TGF-beta 1 were demonstrable over a wide range of concentrations (0.4 to 10 ng/ml). The increased IL-2 protein production was paralleled by a dramatic up-regulation of IL-2 mRNA. In addition, cells precultured with TGF-beta 1 responded with enhanced cluster formation in the secondary cultures. With regard to their phenotype, we observed an increased expression of the alpha E beta 7-integrin human mucosal lymphocyte-1 and of the CD2-restricted epitope CD2R, whereas the expression of CD11a was slightly decreased. In contrast, TGF-beta 1 did not influence the constitutive or activation-induced expression of CD4, CD8, CD45RA, CD45RO, CD25, CD71, CD54, CD58, CD59, and B7. We conclude that TGF-beta 1 supports the generation of human effector cells with a strongly enhanced capacity to respond to subsequent restimulation.

PMID: 7963515 [PubMed - indexed for MEDLINE]

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Transforming growth factor-beta 1 induces antigen-specific unresponsiveness in naive T cells.

Gilbert KM, Thoman M, Bauche K, Pham T, Weigle WO.

University of Arkansas for Medical Sciences, Little Rock 72205, USA.

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Transforming growth factor-beta 1 (TGF-beta 1) is a cytokine with complex immunomodulatory effects including the ability to inhibit the onset or severity of autoimmune disease. This study was designed to test the possibility that one mechanism by which TGF-beta 1 exerts its immunosuppressive effects is by inducing antigen (Ag)-specific unresponsiveness in CD4+ cells. TGF-beta 1 was shown here to inhibit the Ag-specific proliferation of naive CD4+ cells from T cell receptor (TCR) transgenic mice. More importantly, the naive CD4+ cells exposed to TGF-beta 1 and Ag, but not to TGF-beta 1 alone, in primary cultures were unable to proliferate or secrete IL-2 in response to a subsequent Ag challenge following removal of TGF-beta 1 from the cultures. Anti-CD28 mAb partially blocked the Ag-specific inactivation induced by TGF-beta 1 in naive CD4+ cells. The inhibitory effects of TGF-beta 1 on CD4+ cells are not mediated by alterations in APC costimulation since TGF-beta 1 did not inhibit the Ag-induced expression of MHC class II molecules, CD80 or CD86 on splenic APC. Taken together, the results suggest that the immunosuppressive activities of TGF-beta 1 encompass direct induction of Ag-specific unresponsiveness in naive CD4+ cells.

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